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CONTENTS

THE DEMONSTRATION OF NEUTROPHIL ALKALINE PHOSPHATASE ACTIVITY

A. K. LORIMER

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ALKALINE PHOSPHATASE is an enzyme, or closely-related group of enzymes, now known to be present in the cytoplasm of neutrophil leucocytes. This enzyme catalyses the hydrolysis of organic esters of phosphoric acid without being specific in regard to the nature of the alcoholic radical. The enzyme is thus a phosphomonoesterase which reacts optimally, *in vitro*, in the presence of calcium and magnesium ions, in a pH range 9—10, and appears to be identical to the alkaline phosphatase previously demonstrated in serum and other body tissues.

The degree of enzyme activity in the mature neutrophils varies considerably, many leucocytes showing no activity at all, and the overall activity was difficult to assess until the introduction by Kaplow[7] of a scoring system which, whilst being by no means more than crudely quantitative, has yet proved a most useful expedient.

Attention was focused on leucocyte alkaline phosphatase by the observation of many workers,[8 14 15 16] that cytoplasmic phosphatase activity was considerably diminished or even absent in chronic myeloid leukaemia. In leukaemoid reactions concomitant with infections, and in Hodgkin's disease, myelofibrosis, reticulosarcoma, carcinomatosis, polycythaemia vera and some haemolytic anaemias, high levels of alkaline phosphatase activity have consistently been observed. Since in many of these afflictions the leukaemoid reaction may very closely simulate chronic myeloid leukaemia, the estimation of neutrophil alkaline phosphatase activity has become a valuable adjunct to haematological diagnosis. In acute blastic leukaemia and chronic lymphatic leukaemia, varying and non-specific levels of activity have been recorded.

It must be emphasized here that the activity is confined to the mature cells of the myeloid series only, no reaction having been observed until after the metamyelocyte stage. No activity has been found in lymphocytes or monocytes although small amounts of "acid" phosphatase have been noted in lymphocytes.

Only one group of workers has claimed to have observed alkaline phosphatase in eosinophils. In this laboratory, blood smears from an 18 month old child with a total leucocyte count of 75,000 per cubic mm. and an absolute eosinophil count of 56,200 per cubic mm. were exhaustively examined without finding a trace of activity in a single eosinophil. In approximately seventy other smears of normal and abnormal blood and bone-marrow no activity in the eosinophils was seen.

The reaction is of further use in differentiating "secondary" polycythaemia from polycythaemia vera, since it has been demonstrated[1 2 5 8] that a high alkaline phosphatase level in these conditions favours a diagnosis of polycythaemia vera.

HISTOCHEMICAL DEMONSTRATION OF ALKALINE PHOSPHATASE

Since the enzyme splits a suitable phosphate ester into an organic radical and phosphate, the site of enzyme activity may be demonstrated by visualising either the liberated phosphate or the organic component.

In 1939, Gomori[4] and Takamatsu,[12] working independently, introduced an essentially similar technique, finally visualising the phosphate as a black silver salt. The liberated phosphate may also be visualised as black cobalt sulphide and this modification of the original technique has found much favour.

It has already been pointed out that the enzyme is non-specific in regard to the substance with which the phosphate is combined and a large variety of substrates has been employed in *in vitro* experiments. Phenyl phosphates, α- and β-glycerophosphate, adenosine-5-phosphate (the so-called "muscle" adenylic acid), "sugar"-phosphates, and creatine phosphate are some of the phosphates employed. Even pyrophosphates have been used.

In 1944, Menten and her co-workers[10] took singular advantage of this lack of specificity, by using β-naphthol phosphate as the substrate. The β-naphthol liberated by the enzyme was coupled with diazotized *a*-naphthylamine to form an insoluble coloured precipitate at the site of enzyme activity. Many other diazotized amines, which are more stable, have since been used in this admirable technique.[5 7 9 11] The histochemical methods of Gomori-Takamatsu and Menten *et al.*, were originally confined to tissue-sections but may readily be applied to peripheral blood and bone-marrow smears.

Modified Gomori-Takamatsu type reaction

At a pH optimum of about 9.5 the enzyme in the neutrophils acts on a substrate containing organic phosphate and calcium ions, to form calcium phosphate at the site of activity. The calcium phosphate thus formed is converted to cobalt phosphate, using a cobalt salt, and then to black cobalt sulphide with ammonium sulphide.

1. Fix the smears for no longer than thirty seconds in freshly prepared fixative: 10 vols. 40% pure formaldehyde, 90 vols. methyl alcohol.
2. Wash away all traces of fixative with distilled water.
3. Incubate the smears, at 37°C. for 2 hours, in the following substrate mixture, which must be freshly prepared:
   5.0 ml. 10% sodium barbitone.

5.0 ml. 3.2% sodium β-glycerophosphate.
(or 2.5% disodium phenyl orthophosphate. M.W.: 254.01).
5.0 ml. 2.5% magnesium sulphate. $MgSO_4 . 7H_2O$.
7.5% 2.4% calcium nitrate.

Make up to 50 ml. with distilled water. The pH should be 9.6 to 9.8.

4. Discard the substrate mixture and rinse the smears once with a dilute solution of calcium nitrate. (See note below).
5. Place the smears in 2% cobalt nitrate (or any other soluble cobalt salt) for 5 minutes.
6. Wash well with distilled water.
7. Place the smears in a weak (about 1%) aqueous solution of ammonium sulphide. Thirty seconds is adequate.
8. Wash well with distilled water.
9. Counterstain, if desired: 0.5% aqueous safranin O, for about 10 seconds.
10. Wash the smears well and allow to dry in the air.

The fixation is important. All fixatives usually employed either destroy or inhibit enzyme activity to a varying degree. Fixation of the smears in methyl alcohol alone, formalin vapour, or acetone (which distorts the cells), results in marked loss of activity. Smears fixed in the recommended formol-methanol, but at 0—4°C., show slightly increased activity. The fixative, the substrate mixture, and the ammonium sulphide solution are prepared immediately before use. The latter is rather obnoxious. The sodium barbitone and glycerophosphate solutions do not keep well even at 4°C. and should be replaced every month. The glycerophosphate, in particular, may show no visible trace of bacterial decomposition and yet give poor results.

Calcium phosphate is relatively insoluble in water at pH 9 but more soluble at pH 7.5. It has been the author's experience that quite appreciable amounts of precipitated calcium phosphate are lost when the smears are rinsed with the dilute calcium nitrate, in neutral, or often slightly acid, distilled water. Improved staining and less diffusion are obtained if the rinse is prepared as follows: Make up 10 ml. of the 2.4% calcium nitrate to 1 litre with distilled water. Adjust to pH 9.5 by adding N/5 NaOH. The solution is stable.

Any aqueous counterstain that leaves the cytoplasm clear, may be used. The cobalt sulphide precipitate is dark brown, to black. For clear differentiation, therefore, those counterstains which yield a blue-black, or even green, nucleus should be avoided.

The preformed calcium deposits and diffusion difficulties which beset workers with tissue sections hardly occur when working with smears.

Phenyl phosphate is hydrolysed about 4 times faster than glycerophosphate and some build-up of stain at the edge of the nucleus is often observed. If the method outlined is followed carefully, however, no nuclear staining and no diffusion into the nucleus occurs.

Azo-dye coupling technique

Diazotized salts have many commercial applications and are freely available. Not all of them are suitable for enzyme histochemistry and in both editions of his book, Pearse[11] outlines an experiment to determine the most suitable dyes from a number in his possession. In this laboratory, both Fast Blue RR (4-benzoylamino—2 : 5-dimethoxyaniline) and Fast Garnet G (*o*-amino-azo-toluene) have proved effective. Kaplow advocates the use of a propanediol buffer.

Stock Buffer

Dissolve 2.1 grams of Tris (hydroxy methyl) amino methane in 100 ml. distilled water. Store at 0—4°C.

Working Buffer

Dilute 25 ml. of stock buffer and 5.0 ml. of 0.1N HCl to 100 ml. with distilled water.

Technique

1. Weigh out: 20 mg. diazonium salt and 20 mg. substrate (sodium -*a*-naphthyl phosphate).
2. Whilst the smears are being fixed for 30 seconds in 10% formaldehyde in methanol, preferably at 0—4°C., dissolve the salts in 20 ml. working buffer. The preparation must be used immediately.
3. Rinse the slides with tap water.
4. Filter the mixture directly onto the smears and leave for about 10 minutes at room temperature.
5. Wash the slides with running tap water for about 10 seconds.
6. Counterstain with safranin O, if desired.
7. Rinse with water and allow to dry in the air.

The proportion of the two salts and also the buffer is of some consequence and the reagents should be smeared and not just assessed.

There is little to choose from between the two techniques. The intensity and distribution of the stain with the azo-dye technique, which suffers from repetitive weighings, is the same as that obtained, with due care, from the more time-consuming Gomori—Takamatsu method using β-Glycerophosphate.

Mouunting Media

There seems little point in mounting smears if they are to be examined within a day or two of preparation. With both techniques, however,

the stain slowly fades when exposed to the atmosphere and the smears must be mounted if they are to be preserved. The final precipitate obtained from both techniques is soluble in organic solvents and an aqueous mounting medium or polyvinyl pyrolidone is used.

Glycerol-Gelatine[17]

Dissolve 3 grams of powdered gelatine in 50 ml. hot water. Add 20 ml. glycerine with constant stirring. Dissolve 0.2 grams of chrome alum in 30 ml. water with the aid of heat and whilst still warm add to the glycerol-gelatine. Avoid overheating. Adjust the pH to 7.4 with sodium bicarbonate.

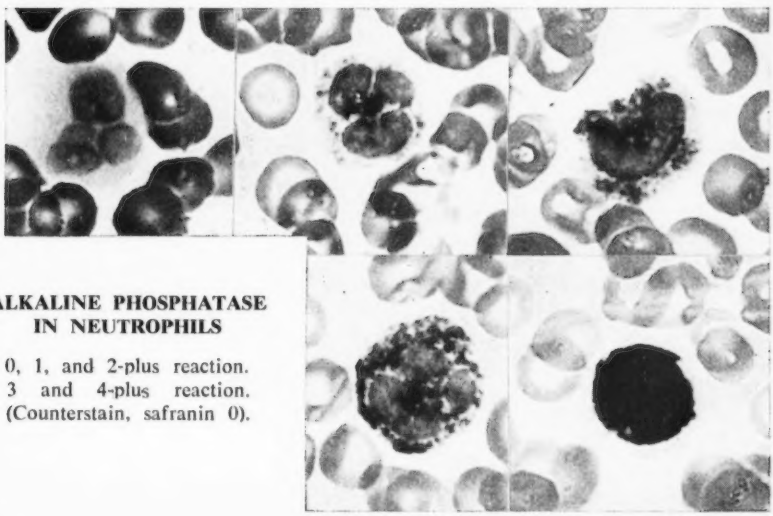
Should the preparation set at room temperature, place in a 37°C. incubator or water-bath until fluid and free from air bubbles.

Polyvinyl Pyrolidone.

Dissolve 50 grams P.V.P. (Plasdone C) in 50 ml. distilled water. Much stirring is required. Stand overnight. Stir in 2 ml. glycerol. This preparation, due to Burstone,[3] tends to be a little too thick and more water can profitably be added.

Scoring Technique

The amount of stained material precipitated in the cytoplasm of the neutrophils is roughly quantitated on a 0 to 4-plus basis, as illustrated in Fig. 1. The 1-plus, 2-plus, and 3-plus categories are, in practice,



ALKALINE PHOSPHATASE IN NEUTROPHILS

0, 1, and 2-plus reaction.
3 and 4-plus reaction.
(Counterstain, safranin 0).

differentiated by the area occupied by the precipitate, rather than by the density of staining. The cytoplasm of the 4-plus cell is a uniform, dense, black. The precipitate in the 3-plus cell, on the other hand, whilst also occupying the whole cytoplasm, is much more open and granular. Four-plus cells are rarely seen in normal smears and an increase in their number suggests some abnormality.

The differential count is performed on one hundred neutrophils, all other cells being ignored. Care must be taken, on account of the weak nuclear staining, not to miss those cells which show no cytoplasmic reaction ("0"). Thin smears, in which the cells are well spread out, are absolutely essential for reliable differentiation on the plus-system.

It is evident that 400 is the highest score that can be obtained from an examination of 100 cells and a "score" of, say, 65 really represents that 65 points were obtained out of a possible 400.

The scores awarded in this subjective technique will vary from one individual to another, being particularly aggravated when thick smears are examined and each observer must establish his, or her, own "normal" range. Kaplow has given his normal range as from 2 to 76 with a mean of 22. Theron, *et al*,[13] report an average score of 180 on 10 healthy Bantu children, and an average of 48 on 8 adult Europeans.

SEMI-QUANTITATIVE BIOCHEMICAL ESTIMATION

In these techniques the enzyme activity of a suspension of leucocytes incubated in a substrate mixture is expressed as the amount of phosphorus or phenol liberated by 10^9 or 10^{10} leucocytes per unit time (usually 15 minutes) of incubation.

The various published procedures for leucocyte suspensions and also for tissue homogenates are by no means comparable with one another. Different substrates have been used with widely different rates of hydrolysis and in many instances no attempt has apparently been made to establish even whether the rate is linear, or not, over the time period and under the conditions employed. It must be borne in mind that a thorough examination of the whole technique is a necessary prerequisite for a quantitative estimation. The author has observed, for instance, that the use of saponin to lyse the leucocytes, can lead to spurious results with at least one method for the determination of alkaline phosphatase.

An even more serious difficulty in interpreting results arises from figures which represent phosphorus liberated from 10^{10} *leucocytes*, as distinct from *neutrophil leucocytes*. Since the alkaline phosphatase activity is confined to the mature neutrophils and the suspension may contain a large proportion of, say, lymphocytes, some form of differentiation is essential. Differential leucocyte counts on smears have been employed as being the obvious answer to the difficulty but this has several well-known limitations as regards accuracy, mostly dependent on the nature of the smears.

A further source of error is the "clumping" of the leucocytes initiated in the sedimentation-flotation separation techniques, making reliable total leucocyte counts exceedingly difficult. Perold (personal communication) has also commented on this difficulty.

Oxalates are toxic to leucocytes. Sequestrene is inhibitory to phosphatase activity, and hence dry heparin is used as the anticoagulant. The author uses 6% Dextran (10 ml. blood, 2 ml. Dextran) to separate the leucocytes from heparinised whole blood. Defibrination alters the "viscosity" of the specimen leading to poor separation with 6% Dextran.

SUMMARY

A practical survey of histochemical procedure for the visualization of neutrophil alkaline phosphatase, with some additional technical notes is presented.

Current biochemical estimations representing activity of the enzyme fall far short of a truly quantitative technique and have not been fully presented.

ACKNOWLEDGEMENTS

I wish to thank Dr. H. B. W. Greig and Dr. J. Metz of the Department of Haematology, S.A.I.M.R., for the facilities extended to me to study the phosphatase enzymes in general, the basic techniques and concepts of which are outlined here. Mr. M. Ulrich produced the photographs.

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'M' IMMUNIZATION

V. F. HIGGS

Natal Blood Transfusion Service, Durban

IN 1927 Landsteiner and Levine injected human blood into rabbits and succeeded in producing an antibody which was subsequently called anti-M. This was the first of a series of discoveries which led to the establishment of a new blood grouping system—the MNSs system.

Although the antigen which was disclosed by anti-M serum is present in some 80% of Europeans, and although it is highly antigenic in rabbits, it seldom produces antibody formation in human beings. Were it not for this fortunate deficiency in antigenicity, the M antigen would be as great a source of anxiety as the Rh factor D. As things are however, the M antigen is ignored in selecting donors for transfusion, and in the serological investigation of pregnant women.

Rarely, however, the MNSs system is involved in haemolytic transfusion reactions and very rarely in haemolytic disease of the new-born. Here is described a case of active immunization by the M antigen.

E. N.—a boy of seven years, suffering from Chronic Collagenosis, was admitted to hospital with Infective Hepatitis. He developed liver failure manifested by coma and a severe haemorrhagic episode during which he was found to have hypoprothrombinaemia, afibrinogenaemia and thrombocytopenia. His blood group was A, Rhesus positive.

On the 12th June, 1960, he was given a transfusion of one pint of group A, Rh positive blood. The compatibility of the donor's blood was tested by the saline, indirect Coombs, and albumin replacement techniques. He received this transfusion well and there were no complications. (The blood transfusion was followed by an infusion of Fibrinogen.)

On 25th June, 1960, a second transfusion was required. On this occasion, difficulty was experienced in finding compatible blood, in that ten group A, Rh positive bloods were tested before a compatible specimen was found.

In the course of investigating the cause of this, several tests were done and many interesting facts were brought to light. These are summarised below:

1. It was found that the blood given on the 12th June, 1960, was now grossly incompatible. This incompatibility was manifested immediately in the saline test.
2. A screen test to identify the abnormal antibody was done. The result pointed to anti-M.
3. The patient's serum was then mixed with seven M positive bloods, all of which it agglutinated and five M negative bloods, none of which it agglutinated.

4. The original donor blood was grouped in respect to the M antigen and found to be positive.
5. The patient's blood was grouped in respect to the M antigen and found to be negative.
6. All the compatible donor bloods were tested in respect to the M antigen and were found to be M negative. (Except the first which as already stated, was M positive.)
7. The antibody had the following characteristics. It was cold, complete, possessed M specificity, and had a saline titre of 64.

This case illustrates a typical difficulty encountered by the laboratory in finding compatible blood, and it also illustrates the important point that a blood specimen which is compatible in that it will not cause a haemolytic transfusion reaction at one time may in fact be incompatible some time later. In short it serves as a reminder of the existence of the M antigen and its occasional antigenicity.

My thanks are due to Dr. Walt and Dr. Winship for clinical details concerning this case, and to Dr. Tanchel (Medical Superintendent, Addington Hospital) and Dr. Ward (Deputy Medical Director, N.B.T.S.) for permission to publish it.

BLOOD pH MEASUREMENT

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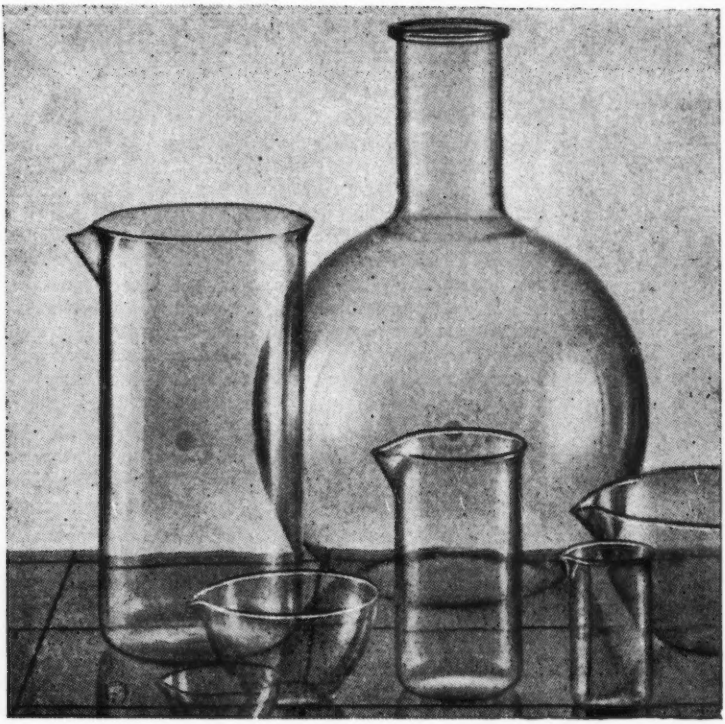
INTRODUCTION

THE EASY AVAILABILITY of inexpensive but efficient flame-photometers and the development of chelating and complexing reagents have made the determination of serum Na, K, Ca together with Cl and bicarbonate a routine procedure in most hospital biochemical laboratories. Frequently the determination of the concentration of these ions in the patient's serum is all that the Clinician needs to carry out the necessary corrective treatment. However, instances do occur where the patient has lost so much of a particular ion or ions that a gross disturbance of acid-base balance results. This is particularly so in the case of young children in whom vomiting, dehydration and respiratory instability are so often a feature of their illnesses. The determination of blood pH is necessary to complete the picture.

SOME EXAMPLES OF BLOOD pH METHODS

A brief survey of the literature on pH measurement reveals a surprisingly large variety of methods; it would appear that no two methods are alike.



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This can be readily understood if it is borne in mind that the majority of published methods are for research purposes and numerous factors must be considered in order to achieve a high degree of accuracy. The choice of method will depend on the accuracy required and the particular conditions under which the measurement is to be made, e.g., operating theatre, bedside or in the laboratory. Methods fall generally into two groups: Colorimetric and Electrometric.

Colorimetric

Of these methods the best known are those of Cullen (1922) as modified by Hawkins (1923), the method of Shock and Hastings (1929) and (1934), and more recently that of Singer *et al* (1955) which is a precise but rather involved modification of the Shock and Hastings technique.

Electrometric

Although the common factor is the use of the glass electrode it is in the electrometric methods that one sees the greatest diversity. A few examples follow:

One of the earliest successful glass electrodes was that described by Stadie *et al* (1931) who also gave details of construction for an electrometer and methods for the preparation of Ag: AgCl reference electrodes. A gas heated bath was used to avoid electrical interference with the electrometer that may result from make and break contacts. The temperature was maintained at 38°C $\pm$ 0.05°C. An overall accuracy of better than 0.01pH was claimed for their technique. A recent modification of this electrode has been described by Anne Chambers (1955).

Holaday (1954) described a glass electrode assembly contained in a portable airbath thermostatically controlled at 37°C. $\pm$ 0.5°C., which would permit serial determinations of arterial blood pH with an accuracy of 0.02 units in the presence of explosive gas mixtures such as would be encountered in the operating theatre.

A complete system of blood pH measurement was described by Murray (1956, i). The principal features being, a new design of electrode assembly holding 0.5 ml. blood the body of which was interchangeable. The electrodes were mounted in a water-bath maintained at 37°C. $\pm$ 0.1°C. Mercury instead of paraffin was used in the handling of blood. In a paper which followed, Murray (1956, ii), the author described a new method for the anaerobic separation of plasma.

Astrup and Schroeder (1956), Wynn and Ludbrook (1957) described electrode assemblies enclosed in water jackets through which water at 38°C. $\pm$ 0.1°C. was pumped.

Wilson (1951) enclosed the whole of the apparatus, solutions, etc. in a specially constructed cabinet which was thermostatically controlled at 37.5°C. and had a glass door with two sleeves, so that the hands could enter the cabinet without the door being opened.

Graig *et al* (1952) measured blood pH anaerobically at room tem-

perature. Blood was collected without oil in a special pipette containing a glass bead and a mixture of Heparin and Sodium Fluoride. The pipette which held 2 ml. could be attached to a syringe by means of a three-way stopcock. Blood was drawn into the syringe and then by means of the stopcock forced out of the syringe into the pipette. The blood after being cooled was pipetted into the electrode assembly.

Straumfjord (1958) and Gambino (1959) described assemblies in which the glass electrodes were maintained at 37.5°C., the reference electrodes being kept at room temperature.

FACTORS INVOLVED IN MEASURING BLOOD pH

The Sample:

(1) *The Effects of exposing the Blood to the Air*

Loss of carbon dioxide from the sample will cause a shift in pH towards the alkalinity. If blood is left unstoppered in a tube and undisturbed after centrifugation no significant change in pH occurs up to two hours. There is however a slight rise in pH in the top few millimeters of the sample, Gambino (1959). It has been shown, Strumfjord (1958), that if blood is gently agitated in a small open beaker at 25°C. its pH rises by approximately 0.01 in 2 minutes, 0.05 in 6 minutes, and by 0.10 in 9 minutes.

Wilson (1951) states that an increase in oxyhaemoglobin of unsaturated blood causes a decrease in pH. This effect is presumably caused by Oxygen-Carbon dioxide exchange between red blood cells and plasma, resulting in an increase in plasma carbonic acid concentration. This phenomenon is important in the case of venous samples.

(2) *Enzymatic Effects*

Once withdrawn from the body and kept for longer periods under anaerobic conditions whole blood becomes more acid in reaction, Havard and Kerridge (1929). This is mainly due to continuous glycolysis which increases the hydrogen ion concentration of the blood. The rate of change depends on the temperature.

Wilson (1951) showed that at 37.5°C. the pH decreased by about 0.05 units per hour while at 4°C. the rate was only one tenth of this. Straumfjord (1958) found the rate of fall of pH at 37.5°C. to be about 0.05 units per hour but found the rate at 25°C. to be about 0.02 units per hour. Bueding and Goldfarb (1941) reported that Sodium Fluoride retarded but did not completely inhibit the formation of hydrogen ions in the blood. Rosenthal (1948) however found the combination of Sodium Fluoride and storage at 4°C. was effective up to 48 hours in preventing significant changes in blood pH.

Haemolysis if present may lead to a drift in pH towards the alkaline side due to the release of carbonic anhydrase. Natelson and Tietz (1956).

(3) *The Collection of Blood Samples*

The blood must be taken under anaerobic conditions and if there is likely to be a delay in making the measurement either the sample must be cooled to 4°C. or Sodium Fluoride must be used.

Blood may be drawn from an arm vein using a syringe with a well fitting plunger. A tourniquet may be used but must be left in position during the sampling, provided the time does not exceed five minutes. Any air that leaks into the syringe must be expelled within 20 seconds. Some authors place the blood under oil but most avoid this, using either specially constructed aparatus, sealed syringes, or vacuum tubes. If capillary blood is used this should be taken using the technique of Shock and Hastings (1934) in this case the use of oil is unavoidable.

For plasma pH, anti-coagulants must be used. Only two need be considered: Potassium Oxalate and Heparin.

Potassium Oxalate: Gambino (1959) states that the use of this anti-coagulant may raise the pH by as much as 0.02 units. Rosenthal (1948) however, states that when the concentration lies between 0.1 and 0.2% no effect can be detected. We have found that a solution of 15% w/v Potassium oxalate when freshly made up with boiled distilled water has a slight alkaline reaction and must be brought to pH 7.0 with 0.1N Oxalic Acid. An aliquot of the Potassium oxalate solution is dried onto the inner wall of the sample tube.

Heparin: This appears to have no effect on blood pH but has the disadvantage of being effective for only a short time.

(4) *Effects of Temperature*

The pH of whole blood and plasma is dependent upon its temperature there being a linear rise of approximately 0.015 units for each degree Centrigade below 38°, Rosenthal (1948). This change in pH with change in temperature is in no way related to the changes in the constants of the measuring instruments but is a real and reversible change in pH due to the decrease in dissociation of organic acids at the lower temerature. Dill *et al* (1937) have shown that the temperature coefficient varies with the reaction of the blood. The more acid the blood the less the change per degree.

The temperature coefficient of plasma varies with the temperature of separation from the red cells (temperature of centrifugation) being approximately —0.009/°C. and —0.016/°C. for separation at 37°C. and 4°C. respectively, Gambino (1959). The effect of temperature on glycolysis was dealt with under "Enzymatic Effects".

INSTRUMENTAL FACTORS

Only those factors which affect the actual measurement of blood pH and are under the direct control of the operator will be dealt with here.

(1) Temperature

This is the most important factor and the one mainly responsible for the diversity in apparatus.

The calomel electrode usually employed with the glass electrode assembly is very temperature dependent. This effect is greatest with electrodes that are filled with saturated Potassium Chloride solution and much less with electrodes filled with 0.1N Potassium Chloride. Most pH meters have a device which compensates for the alteration in potential of the reference electrode at the various temperature levels. Where such a device is not fitted or when a reference electrode of a different type is used, a calibration curve is essential. Two points must be stressed: (*a*) The temperature of the reference electrode must be known with certainty; (*b*) The temperature of the reference electrode must remain constant throughout the series of measurements beginning with the standardization procedure.

The glass electrode is a little less affected by the temperature changes; its electrode is sealed within a glass envelope and the assembly, unless relatively large, fairly rapidly equilibrates with the temperature of the test sample. However, temperature gradients across the glass membrane must be avoided. Holaday (1954) states that drifts exceeding 0.10 units were observed when the glass electrode was rinsed with water of various temperatures between buffer readings. These temperature effects are additional to those which occur in the test solution due to changes in dissociation of the solutes in the sample.

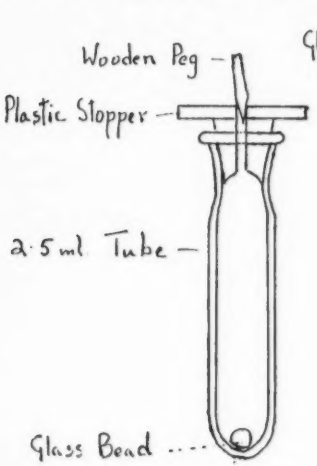

Fig. 1

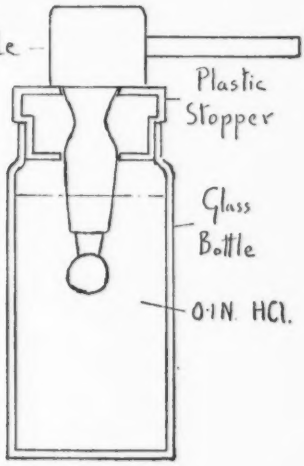

Fig. 2

(2) The Salt Bridge

It is essential that the concentration of the electrolyte is what it is supposed to be, e.g. if saturated potassium chloride is used, then the solution must be saturated a the temperature of the measurement. Air bubbles and other obstructions must be avoided. Saturated potassium chloride solution is used because of its low contact potential therefore any contamination or change in saturation may lead to large errors.

(3) Position of Electrodes

The relative position of the electrodes must be fixed and sufficient space must be available to allow free diffusion of the solution from the reference electrode, e.g. not less than $\frac{1}{8}$" between tip of electrode and the bottom of the sample beaker.

(4) Cleanliness of the Apparatus

All the external surfaces of the electrodes, supports, leads and terminals should be kept dry, otherwise conducting paths may be formed which allow leakage of the small charges that are being measured. All electrical connections should be kept free of dirt and corrosion. The insulating material of leads, etc., should be inspected periodically for faults.

A glass electrode that suddenly develops an abnormally large asymmetry potential has usually an altered outer surface. This is almost always caused by either an abrasion or contamination of the surface. The former is avoided by careful handling and the latter by an adequate cleaning procedure and prevention of contamination. Liquid paraffin is a prime offender in this regard and is best avoided if other ways can be adopted for the anaerobic handling of blood.

(5) Suspension Effect

Severinghaus *et al* (1956) showed that whole blood pH read approximately 0.01 unit lower than its true plasma pH. This they put down to the presence of solid particles (blood cells) at the glass membrane which interfered with the diffusion of ions. On the other hand Gambino (1959) found a variable difference that may have a maximum of 0.10 pH unit, the plasma always being more alkaline than the blood, but found no such difference in patients completely at rest. This phenomenon needs further investigation before whole blood pH can be accepted as suitable for pCO_2 computation.

(6) Linearity of the Instrument

The pH assembly should be checked from time to time using at least three buffers one of which should be a primary standard.

ACCURACY AND PRECISION

The definition of accuracy and precision should be kept in mind when reading literature on pH Methodology.

Precision

There is not usually any difficulty in measuring the precision of the method provided adequate samples of blood or plasma can be obtained. The method of choice would be to obtain samples in duplicate and use the difference between duplicates to calculate the standard deviation. Estimates of precision based on replicate measurements on buffer solutions are not valid, plasma or blood only should be used. The attainment of precision better than 0.02 is not easy.

Accuracy

It should be noted that the basic function of a pH meter is to measure the difference in E.M.F. between two solutions under practical conditions. One of the solutions must be of known pH and from the difference in E.M.F., the pH of the unknown is computed. Two measurements are thus necessary.

For practical purposes the following "Operational" definition of pH is generally accepted.

$$pH = pH_s + \frac{(E—E_s)F}{2.3026RT}$$

where pH_s is the value of the standard, E_s the E.M.F. of the standard, E the E.M.F. of the unknown, T the absolute temperature, R the gas constant and F the Faraday. The whole system of pH measurements depends on the existence of a Scale of pH standards.

It is likely that at present no exact estimate of the accuracy of blood pH measurements can be made. This is simply because there is no suitable standard. The buffer solutions normally used as standards differ markedly in composition and physical properties from blood and plasma. This does not mean that methods generally are inaccurate but rather that one must be cautious in accepting claims that a particular method is accurate to a definite degree. The accuracy of a method is best judged by an appraisal of the methodis adopted to eliminate known sources of error.

STANDARD BUFFER

The British Standard pH Scale (British Standard 1647 (1950)) is applicable only to aqueous solutions at temeratures between 0 and 60°C. A single primary standard is adopted and is a 0.05M solution of Potassium Hydrogen Phthalate to which is assigned the value of pH 4.01 $\pm$ 0.01 at 25°C. A further six buffer solutions are given as secondary standards.

Certified samples of buffer substances can be obtained from the National Bureau of Standards, Washington, U.S.A. from which reference solutions of reproducible pH can be made. Reference should be made to "Standardization of pH Measurements made with the Glass Electrode" N.B.S. letter Circular L.C. 993 (1959).

A SIMPLIFIED METHOD OF BLOOD pH MEASUREMENT

The following procedure has been in use in this laboratory for over a year. It is reliable, uses readily available equipment and requires no great skill once the system is established.

When 12 blood samples were taken in rapid succession from a Heart-Lung machine during an operation the subsequent measurements had a standard deviation of 0.018.

Apparatus

(1) Beckman Model G pH meter.

(2) Beckman electrode assembly No. 40313 using Reference electrode No. 39270.

(3) A special blood pH tube. This consists of a plastic stoppered test tube of capacity 2.5 to 3.0 ml. The stopper has a $^1/_{16}$" hole drilled down its centre. The hole is plugged with a wooden peg. The interior of the tube is coated with an anti-coagulant and an enzyme inhibitor. No oil is used. See Fig. 1.

(4) Potassium oxalate 15% w/v aqueous solution adjusted to pH 7.0 with 0.1N Oxalic Acid.

(5) Sodium Fluoride 4.5% w/v aqueous solution adjusted to pH 7.0. *Note:* 0.05 ml. of each solutions (4) and (5) are added to the pH tube and dried in an oven at 60°C. A glass bead is then added.

(6) Beckman Buffer concentrated solution No. 3501 pH 7.02 at 20°C.

(7) Phosphate buffer M/15 pH 7.83 at 20°C.

(8) Beckman saturated Potassium Chloride Solution No. 3502.

(9) 0.9% w/v Sodium Chloride Solution in a large aspirator.

(10) Dilute Teepol 1 in 15 v/v aqueous solution.

(11) Strong Teepol 1 in 3 v/v aqueous solution.

(12) Two 10 ml. hypodermic syringes for the buffer solution fitted with No. 20 needles.

(13) One 2 ml. hypodermic syringe to draw off plasma fitted with No. 20 3" spinal needle.

(14) 0.05 M Potassium Hydrogen Phthalate B.D.H. Analar.

Procedure

Blood is taken as previously described. The pH tube is filled to the brim with blood and the plastic stopper inserted so that a little blood is ejected through the hole. The wooden peg is replaced making an air-tight fit. The tube is then inverted several times and placed in a beaker of water at room temperature. Note the temperature and centrifuge for ten minutes, then place the tube back into a beaker of water at room temperature. Meanwhile set up the instrument using pH 7.02 buffer as described in Beckman Operating Instruction No. 204A and check the following points:

1. Instrument warmed up and stable.
2. Range switch to pH.
3. Temperature compensator set correctly, i.e., room temperature.
4. Instrument in balance with controls 1 and 2.
5. Reference Electrode full to just below side arm.
6. Reference Electrode stopper removed.
7. Reference Electrode lead connected to lower terminal.
8. Glass Electrode lead connected to upper terminal.
9. Beaker filled with saturated KCL solution.
10. Electrode tips $^1/_{16}$" apart.
11. Electrode tips $\frac{1}{8}$" from bottom of beaker.
12. All connections clean, dry and firm.
13. Compartment door properly closed.

Repeat the standardization with pH 7.83 buffer. This reading should be within 0.02 of its expected value. If it is not then the standardization procedure should be repeated and if necessary both buffers should be checked against the primary standard at pH 4.01. It is obviously better to check the buffers from time to time to ensure that they are always ready for use.

The glass electrode and chamber are then washed in running water at room temperature, excess water being removed with filter paper. Plasma is drawn into the 2 ml. syringe by inserting the 3" needle into the hole in the stopper. Remove air bubbles, if any, without delay or fill the dead space of the syringe with freshly boiled distilled water. Inject the plasma into the chamber through the rubber stopper flushing out all air bubbles. Half a drop of plasma must protrude from the side arm. Place electrode in position on the door of the pH meter and take the reading.

Correct the pH for temperature effect using the formula of Rosenthal (1948).

Blood pH_{38} = Plasma pH — 0.0118 (38-tm) — 0.0029 (38-tc)
where tm = temperature of plasma pH measurement

tc = temperature at which blood was centrifuged.
Usually tm = tc = room temperature.

Method of Cleaning and Storing Electrode

The glass electrode should be well washed in running normal saline from the aspirator, this cannot be overdone. Blot with filter paper and place in strong Teepol solution for 15 minutes. Rinse for 2 or 3 minutes in dilute Teepol, give a quick wash with saline, blot and store in 0.1N HCl until further use. Just before use, the glass electrode should be well washed in water and immersed in buffer at pH 7.02.

Fig. 2 shows our method of storing the glass electrodes. Care should be taken to see that the bulb of the electrode is completely immersed.

SUMMARY

The main principles of measuring blood pH are discussed.

A few samples of methods are given and a simple practical procedure is presented.

It is hoped that this paper will be of some assistance to those who intend to introduce this determination into their laboratory routine.

ACKNOWLEDGEMENTS

I wish to thank Dr. D. McKenzie for permission to publish this paper and his help and constant interest; Dr. A. Bull for the encouragement and co-operation he gave; Mrs. D. Melnick and Miss B. Henrichsen for the typing and help with the clerical work, and Prof. J. E. Kench for his constructive criticism of the manuscript.

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BOOK REVIEWS

Anaerobic Bacteriology in Clinical Medicine. A. Trevor Willis. 163 pages. Butterworth & Co. (Africa) Ltd., 33-35 Beach Grove, Durban, Natal. 34s. 6d. plus 1s. 3d. delivery charge.

This publication fills a long-felt need for a concise work on an important subject which, only too often is neglected in some laboratories.

The author covers both the technical and clinical aspects with emphasis on the best methods of isolating and identifying anaerobic organisms from various sites.

This book should be of inestimable value to the clinician as well as to the laboratory worker.

J.R.H.

Handbook of Haematology and Blood Transfusion Technique. J. W. Delany. 311 pages. Butterworth & Co. (Africa) Ltd., 33-35 Beach Grove, Durban, Natal. 51s. 9d. plus 2s. 0d. delivery charge.

This book, written by a medical laboratory technician, who is also an examination assessor will be found to be valuable addition to literature an examination assessor will be found to be valuable addition to literature on this subject.

The section on haematology is well covered, ranging from the basic techniques to the elucidation of the aetiology of blood disorders, dealing also with genetics and the use of radio-isotopes.

The section on blood transfusion technique includes cross-match in the method of packing cells and the preparation of plasma.

The author covers extensively the various blood groups, transmission of disease, reactions and the practical approach to the blood bank, storage and refrigeration.

This publication, which includes a number of coloured plates, tables and diagrams will be most useful to student technologists and haematologists.

J.R.H.

An Introduction to the Anaesthesia of Laboratory Animals. Phyllis G. Croft, PH.D., M.R.C.V.S. U.F.A.W., 7a Lamb's Conduit Passage, London, W.C.1. 3s. 6d. 31 pages.

Dr. Croft, as a UFAW Research Fellow, is carrying out research at the Royal Veterinary College on the subject of anaesthesia, with a particular concern for the ways of guarding against the spurious appearance of anaesthesia which can occur in a conscious animal under certain conditions.

Her booklet is intended to assist those who are not already familiar with the administration of anaesthetics to laboratory animals, and covers the subject very well indeed.

It describes the best methods and anaesthetics which should be used for the different animals under various conditions, pointing out the advantages and disadvantages of each. It also describes the emergency measures to adopt in cases of accidental overdose of anaesthetic and the tests for reflexes to assess depth of unconsciousness.

A very useful addition to laboratory libraries.

J.R.H.

The South African Journal of Laboratory and Clinical Medicine. Published by the Medical Association of South Africa, P.O. Box 643, Cape Town. Annual subscription: 25s. 0d.

The Journal deals with laboratory medicine and in bringing it to the attention of our readers, we feel that they will find something of interest in its pages. A typical issue contained.:—

Cutaneous Porphyria: L. Eales, M.D., M.R.C.P.

Observation on the Role of Immune Antibody in Protection in Enteric Infections: Kenneth C. Watson.

Catalytic Hydrolysis of Urinary 17-Ketosteroid Glucuronides by Amberlite IR-120: S. M. Joubert.

'n Ondersoek na die Invloed van Monasietstof op die Longe van Rotte na Intratrageale Inspuiting: B. F. Thiart en F. M. Engelbrecht.

Lysogeny in Genus Proteus: I. Incidence of Lysogenic Strains: J. N. Coetzee and T. G. Sacks.

CORRECTION

OPTICAL INSTRUMENTS (Pty.) LTD.

WE WISH to draw attention to an unfortunate error which crept into Messrs. Optical Instruments advertisement in the June issue of this Journal. Messrs. Optical Instruments, of course, operate from Johannesburg and Cape Town. Correct addresses should read:—

Dept. Med. P.O. Box 1561, Johannesburg.
P.O. Box 4051, Cape Town.

We apologise to Messrs. Optical Instruments for any inconvenience which the error may have caused.

AMENDMENTS TO THE CONSTITUTION

AT THE REQUEST of Council and for the information of members we are re-publishing a complete list of amendments which have been made to the Constitution since 1957.

Para. 5 b. Members:

Membership of the Society is open to any person who has completed the requirements of the Rules and Regulations regarding the Registration of Medical Technologists as gazetted from time to time by the South African Medical and Dental Council.

Para. 5 c. Student Members:

A Student Member of the Society is deemed to be any person who has not yet completed the requirements of 5 b above.

Para. 5 d. Affiliated Members:

The Society may elect as Affiliated Members any persons interested in Medical Technology. The approved Affiliated Members shall not be allowed to vote.

(Notwithstanding, any person who has attained membership of the Society prior to the publication of these amendments shall in no way be prejudiced.)

Para 8 i. Management.

Insert, after the word Society in line 3, the following:—

The persons so elected must be Registered Medical Technologists, or be eligible for registration as such, with the South African Medical and Dental Council.

Para 11 ii. Annual General Meeting.

Delete the word "voting" from line 6 of this clause.

Para 12. Finance.

In sub-section iii substitute "one quarter" for the existing words "one third".

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Acetyl Suspension and Syrup Dosage		
Weight	First day	Thereafter
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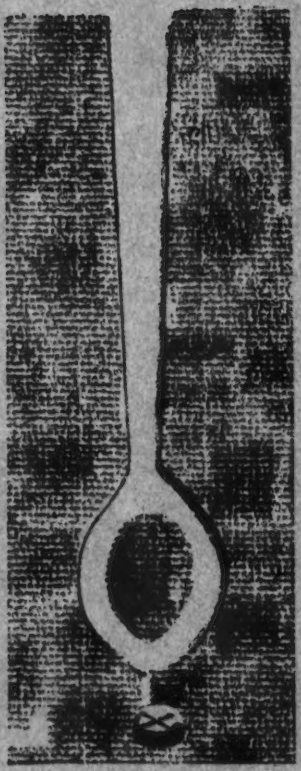
40 Lb.	2 Tsp.	1 Tsp. daily
80 Lb. and over	4 Tsp.	2 Tsp. daily



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